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Relation between K469E gene polymorphism of ICAM-1 and recurrence of ACS and cardiovascular mortality

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ABSTRACT

Objective: To explore the relation between K469E gene polymorphism of intercellular adhesion molecular-1 (ICAM-1) and the recurrence of ACS and cardiovascular mortality. **Methods:** A total of 185 patients with ACS hospitalized in Department of Cardiology in our hospital from Sep 2007 to Sep 2008 were selected as objectives. Polymerase chain reaction was used to analyze K469E gene polymorphism of ICAM-1. According to the genotypes, they were divided into two groups: group with K allele (KK+KE) and group without K allele (EE). The two groups were followed up prospectively for five years and blood lipid, blood pressure, blood glucose, recurrence and death of ACS were collected when the patients left hospital. The relation between ICAM-1 gene polymorphism and the recurrence of ACS and cardiovascular mortality was analyzed by Logistic regression. **Results:** After long-term follow-up, it was found that ACS recurred on 71 cases (38.4%) and 10 cases died, among which 3 cases died of cardiovascular disease. The recurrence of ACS and cardiovascular mortality in group with K allele were remarkably higher than that in group without K allele ($P<0.01$). After multivariate Logistic regression adjusted ages, gender, weight indexes, TC, LDL-C, TG, smoking, drinking, family history of cardiovascular disease, history of hypertension and the severity of coronary artery disease, the risks of ACS recurrence and cardiovascular mortality in group with genotype KK+KE was 3.31 and 3.53 times of those in group with genotype EE respectively ($P<0.01$). When the independent variable of hypertension was introduced in regression analysis, the risks of ACS recurrence and cardiovascular mortality in group with K allele both decreased ($P<0.05$). When the independent variable of HDL-C was introduced, different genotypes of ICAM-1 weren't relevant with ACS recurrence and cardiovascular mortality ($P>0.05$). **Conclusions:** K469E gene polymorphism of ICAM-1 was related to ACS recurrence and cardiovascular mortality, K allele probably an independent risky factor and hypertension and to which the level of HDL-C were closely related.

1. Introduction

Acute myocardial infarction and unstable angina pectoris are both referred to as acute coronary syndrome (ACS), which is currently one of the most common diseases that are harmful to human life and health. So far, it is considered that the important mechanism of ACS pathogenesis is that unstable plaque of coronary atherosclerosis falls apart and consequently leads to the formation of thrombus that blocks the lumen[1]. Since the tissues of plaque contain a lot of inflammatory cells and activated inflammatory cells can secrete excess pro-inflammatory cytokines which make the plaque of coronary artery easy to fall apart, lessening inflammation is helpful to make plaque stable, prevent and postpone the occurrence and development of ACS.

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Intercellular adhesion molecule-1 (ICAM-1) mediates the activation of endothelial cells, triggers inflammatory reactions and contributes to the aggregation of a lot of inflammatory cells, which plays an important role in the formation of plaque. ICAM-1 gene has K469E polymorphism and many foreign studies reported that K469E polymorphism was closely related to stability of the plaque of coronary atherosclerosis[2,3]. The instability of plaque can lead to the recurrence of ACS and cardiovascular mortality. In the study, PCR technique was used to detect gene polymorphism. The value of predicting the recurrence of ACS and cardiovascular mortality by ICAM-1 gene polymorphism was discussed to provide basis for the individual prevention and treatment of high-risk patients.

2. Materials and methods

2.1. General data

A total of 185 patients with ACS hospitalized in Department of Cardiology in our hospital from Sep 2007 to Sep 2008 were selected, including 128 male cases and 57 female cases, all of whom were in accordance with the diagnostic standards of ACS drafted by American College of Cardiology in 2002[4] and were confirmed by coronary arteriongraphy. All the patients were Han people and those with severe liver, renal insufficiency, autoimmune disease, tumor and acute or severe chronic infectious diseases were excluded. All the patients had no genetic connections and signed the informed consent and were approved by ethics committee before experiment.

2.2. Method

2.2.1. Sample collection and DNA extraction

Three mL peripheral venous blood was taken from patients with empty stomach early in the morning. EDTA-K_2 anticoagulant was used to anti-freeze. White blood cells were separated by Ficoll solution. DNA was extracted by Genomic DNA Extraction Kit (Beijing Bai Taike Biotechnology Co., Ltd.).

2.2.2. Detection of K469E polymorphism of ICMA-1 gene

PCR-RFLP method was used to detect K469E polymorphism of ICMA-1 gene. ①Primer synthesis: the primer was synthesized by Beijing SaiBaiSheng Co. Ltd. The upstream primer and downstream primer were respectively 5′-GGAACCCATTGCCCGAG-C-3′ and 5′-GGTGAGGATTGCATTAGGTC-3′. ②PCR reaction system: total volume 25 μL, including 20 pmol upstream primer, 20 pmol downstream primer, 5.0 μL template DNA, 2.5 μL 10×PCR buffer solution, 2.0 μL 2.5 mmo1/L dNTPs, 1.25 μL *Taq* DNA polymerase and finally complemented by sterile double distilled water to 25 μL. ③The conditions of PCR amplification: being pre-denatured for 5 min at 94 ℃, denaturation for 45 s at 94 ℃, annealing for 60 s at 61 ℃ and being stretched for 60 s at 72 ℃, the above four steps were repeated for 35 times; being stretched for 5 min at 72 ℃. ④Restriction enzyme digestion and electrophoresis: 10 μL PCR amplification products were collected, mixed with 4 urestriction enzyme BstU I (British Biolabs Company) and hatched for 5 h at 60 ℃. After all the reactions, enzyme-digested products were examined by 3% agarose gel electrophoresis. Then the products were stained by ethidium bromide and the results of genotypes were read by gel electrophoresis imaging instrument.

2.2.3. Follow-up visit

After patients left the hospital, the smoking, drinking, blood fat, blood sugar, history of diabetes mellitus, family history of stroke, history of hypertension, recurrence and death events were followed up for 5 years through telephone and standard questionnaire.

2.3. Statistical methods

All the data were analyzed statistically with software SPSS 17.0, comparisons of indexes such as characteristics of different genotypes of demography, smoking, drinking, blood fat, blood sugar, history of diabetes mellitus, family history of stroke, history of hypertension and so on were tested by t and χ^2. Gene polymorphism of ICAM-1 and the relation between other dangerous factors and the recurrence of ACS and cardiovascular mortality were analyzed by gradually non-conditional Logistic regression. Relative risk was expressed by oddsrations (*OR*) and 95% confidence intervals (*CI*). The difference had statistical significance as $P<0.05$.

3. Results

3.1. Analysis of ICAM-1 genotype

Mutation happened at exon 6 A→G site of ICAM-1 gene, which directly affected the change from 469 amino acid K (lysine coded by the gene) to glutamic acid E. The length of ICAM-1PCR amplification fragment was 223 bp. According to the state of fragments, there were three genotypes: genotype KK (only one band 223 bp), genotype KE (three bands: 223 bp, 136 bp and 87 bp) and genotype EE (two bands: 136 bp and 87 bp). See Figure 1.

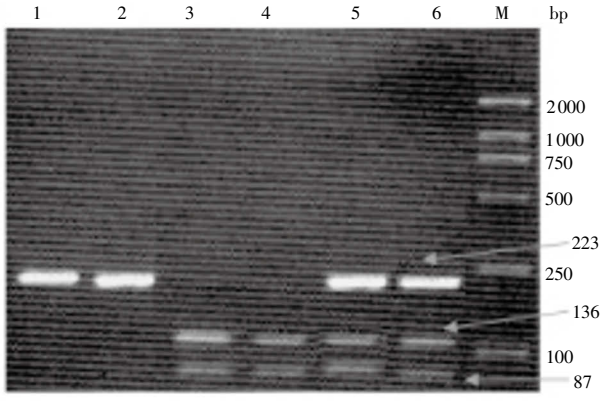

Figure 1. Electrophoretogram of ICAM-1 K469E genotype.
Note: M: DL 2000 DNA marker; 1, 2: Genotype KK; 3, 4: Genotype EE; 5, 6: Genotype KE.

3.2. Comparison of clinical features in group with K (KK+KE) allele and group without K allele (EE)

During the five-year follow-up visit, ACS recurred on 71 cases (38.4%) and 10 cases died, among which 3 cases died of cardiovascular disease. The recurrence of ACS, cardiovascular mortality, history of diabetes mellitus and the level of HDL-C were all remarkably higher than those in group without K allele (EE) ($P<0.01$). There was no statistical significance in the difference of constituent ratio of ages and genders in the two groups, weight index, TC, LDL-C, TG, smoking, drinking, family history of cardiovascular diseases, history of hypertension and the scores of the severity of coronary artery disease ($P>0.05$). See Table 1.

3.3. Analysis of correlation between K469E polymorphism of ICAM-1 gene and the risk of ACS recurrence

ICAM-1 gene, age, gender, weight index, TC, LDL-C, TG, smoking, drinking, family history of cardiovascular diseases, history of hypertension and the severity of coronary artery disease were considered as independent variables and whether ACS recurred or not was considered as a dependent variable. The results of Logistic regression analysis showed that the risk of ACS recurrence in group with K allele was 3.31 times of that in group without K allele (*OR*=3.31, 95%*CI* was within 1.46-8.02, $P<0.01$). When the independent variable of hypertension was introduced in regression analysis, the risk of ACS recurrence in group with K allele decreased (*OR*=3.02, 95%*CI* was within 1.01-9.07, $P=0.03$). When the independent variable of HDL-C was introduced, different genotypes of ICAM-1 weren't relevant with ACS recurrence ($P=0.24$). See Table 2.

Table 1
Comparison of clinical features of different genotypes of ICAM-1 gene K469E (mean±sd).

Clinical features	Group with genotype EE (*n*=1169)	Group with genotype KK+KE (*n*=69)	Value of *t* or χ^2	*P* Value
Age/Year	58.60±9.70	59.10±10.50	0.33	>0.05
Male/case (%)	79(68.1)	49(71.0)	0.06	>0.05
Weight index	24.30±3.70	24.60±4.00	0.52	>0.05
TC (mmol/L)	4.84±2.35	5.06±3.15	0.54	>0.05
HDL-C (mmol/L)	0.98±0.37	1.13±0.42	2.53	<0.05
LDL-C (mmol/L)	2.99±0.96	3.05±0.78	0.44	>0.05
TG (mmol/L)	1.90±0.60	1.85±0.72	0.51	>0.05
Smoking/case (%)	60(51.7)	32(46.4)	0.30	>0.05
Drinking/case (%)	41(35.3)	27(39.1)	0.13	>0.05
Family history of cardiovascular disease/case (%)	13(11.2)	5(7.2)	0.39	>0.05
History of hypertension/case (%)	72(62.1)	38(55.1)	0.61	>0.05
History of diabetes mellitus /case (%)	15(12.9)	19(27.5)	5.22	<0.05
Severity of coronary artery disease (Gensini score)/point	61.43±18.57	62.25±17.30	0.29	>0.05
ACS recurrence/case (%)	37(31.9)	34(49.3)	4.81	<0.05
Cardiovascular Death/case (%)	16(13.8)	20(29.0)	5.44	<0.05

Table 2
Correlation between K469E polymorphism of ICAM-1 gene and the recurrence of acs and the risk of cardiovascular death.

	Ratio of the risk of ACS recurrence *OR* (95%*CI*)			Ratio of the risk of cardiovascular death *OR* (95%*CI*)		
	Genotype EE	Genotype KK+KE	*P* Value	Genotype EE	Genotype KK+KE	*P* Value
A	1.0	3.31(1.46-8.02)	0.00	1.0	3.53((1.54-8.47)	0.00
B	1.0	3.02(1.01-9.07)	0.03	1.0	3.16(1.09-9.52)	0.02
C	1.0	1.26(0.86-4.79)	0.24	1.0	1.45(0.69-3.08)	0.32

Note: A: the results based on the independent variables (ICAM-1 gene, age, gender, weight index, TC, LDL-C, TG, smoking, drinking, family history of cardiovascular diseases, history of hypertension and the scores of the severity of coronary artery disease); B: results after history of hypertension was added in variables; C: results after history of hypertension and HDL-C were added in variables.

3.4. Analysis of correlation between K469E polymorphism of ICAM-1 gene and the risk of cardiovascular death

The results of Logistic regression analysis showed that the risk of cardiovascular death in group with K allele was 3.53 times of that in group without K allele (*OR*=3.53, 95%*CI* was within 1.54-8.47, *P*<0.01). When the independent variable of hypertension was introduced in regression analysis, the risk of cardiovascular death in group with K allele decreased (*OR*=3.16, 95%*CI* was within 1.09-9.52, *P*=0.02). When the independent variable of HDL-C was introduced again, different genotypes of ICAM-1 weren't relevant with cardiovascular death (*P*=0.32). See Table 2.

4. Discussion

ICAM-1 belongs to the super-family of immunoglobulin, composed of 507 amino acids and expressing extensively on the surface of endothelial cells, macrophage and activated leukomonocyte. It mediates the adhesion of vascular endothelial cells and white blood cells through combining with function associated antigen of its ligand lymphocyte and plays an essential role in the inflammatory mechanism of atherogenesis[5-7]. Its coding gene is located in p13.3-p13.2 of human chromosome 19 with the total length of 15.5 kb, including 7 exons and 6 introns. The sixth exon has a common polymorphism (A→G), influencing 469th codon (AAG→GAG) and finally causing lysine to glutamic acid. And such gene mutation may affect the Plasma concentration and activity of ICAM-1[8,9]. Currently, Many studies in domestic and abroad reported that K496E polymorphism was related to the occurrence of myocardial infarction and stroke[10-12]. However, there was no report about the study of K496E polymorphism and the recurrence and risk of death of heart or brain vessels disease.

After five years' follow-up visit of 185 ACS patients, it was found that ACS recurred on 71 cases (38.4%) and 10 cases died, among which 3 cases died of cardiovascular disease. The recurrence of ACS and cardiovascular mortality in group with K allele (KK+KE) were remarkably higher than that in group without K allele (EE) (*P*<0.01), indicating K allele increased the risk of the recurrence and death of ACS. It alsofurther analyzed whether interactions between K469E polymorphism of ICAM-1 gene and age, gender, hypertension, diabetes mellitus and abnormal blood lipids or not. When ICAM-1 gene, age, gender, weight index, TC, LDL-C, TG, smoking, drinking, family history of cardiovascular diseases, history of hypertension and the severity of coronary artery disease were considered as independent variables and whether ACS recurred or not was considered as a dependent variable, the results of Logistic regression analysis showed that the risk of ACS recurrence in group with K allele was 3.31 times of that in group without K allele (*OR*=3.31, 95%*CI* was within 1.46-8.02, *P*<0.01). If cardiovascular death was considered as dependent variable, the results of Logistic regression analysis showed that the risk of cardiovascular death in group with K allele was 3.53 times of that in group without K allele (*OR*=3.53, 95%*CI* was within 1.54-8.47, *P*<0.01), indicating that K allele may be one of the independent and dangerous factors of ACS recurrence and cardiovascular death[13]. When the independent variable of hypertension was introduced in regression analysis, the risks of ACS recurrence and cardiovascular mortality in group with K allele both decreased (*P*=0.03, *OR*=3.02; *P*=0.02, *OR*=3.16). When the independent variable of HDL-C was introduced again, different genotypes of ICAM-1 weren't relevant with ACS recurrence and cardiovascular mortality (*P*=0.24, *OR*=1.26; *P*=0.32, *OR*=1.45). The above results revealed that K469E polymorphism of ICAM-1 may contribute to the recurrence of ACS and cardiovascular death by affecting hypertension and HDL-C.

The study found that the difference had no statistical significance in the comparison of the severity of coronary artery diseases in two groups (*P*>0.05), indicating that there was no obvious relation between ACS recurrence, cardiovascular death and the severity of coronary artery diseases. The pathogenesis of ACS was caused by unstable atherosclerotic plaque falling apart and consequently led to the thrombogenesis. And plaques often fell apart at the moderately narrow places in coronary artery, so the occurrence of ACS mainly depent on the stability of plaques rather than the degree of coronary artery stenosis[14,15]. ICAM-1 gene probably affected ACS recurrence and cardiovascular death through triggering the unstability of plaques rather than aggravating the severity of coronary artery[16]. The study also found that there were relatively more cases with diabetes mellitus in group with genotype KK+KE (27.5% *vs.* 12.9%, *P*<0.05). Some studies[17] found that diabetes mellitus and secondary insulin resistance were dangerous factors of ACS recurrence, but whether the higher risk of ACS recurrence in group with K allele is related to the history of diabetes mellitus needs further study.

In conclusion, ICAM-1 K469E polymorphism may be one of the dangerous genetic factors of ACS recurrence and cardiovascular death and the pathogenesis was related to hypertension, HDL-C and stability of plaque. But ACS was a disease of multi-factorial inheritance and closely related to

environmental factors. The interactions of gene to gene and gene to environment need further reaearch[18,19]. Besides, ethnic difference of study objects, sample capacity and detail methods as well as diagnostic standards are main factors of the occurrence of heterogenicity[20]. Therefore, such a conclusion needs more comprehensive samples to verify. If the results can be ensured, it would be remarkably helpful for the individual prevention and treatment.

Conflict of interest statement

We declare that we have no conflict of interest.

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